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Differentiation of root canal morphology – a review of the literature

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ABSTRACT: The morphological diversity of the inner anatomy of root canals in human permanent teeth is an issue mainly described in endodontics research. In recent years, it is a relatively new point in anthropological studies because it varies by sex, geographic and ethnic circumstances. An unusual number of root canals or their specific systems can differentiate populations around the world. The multiplicity of available methods for detecting root canal systems helps to better understand their complexity; however, most cannot be applied to anthropology due to limitations that occur in the bioarcheological material. Thorough knowledge of the heterogeneity and internal anatomy of the root canals supplies numerous classification systems proposed and improved over the years by many authors. A limited number of studies in the anthropological literature and future research will shed light onto the internal tooth morphology in historical populations. The aim of this paper is to describe various classification systems of root canals applied in endodontics. However, due to the often poor state of preservation and damage of archaeological derived remains, it seems that the best method is the approach that is the simplest and least complicated.

KEY WORDS: root canal, tooth morphology, classifications, C-shape

Introduction

Researchers have recently shown that the root canal anatomy of permanent teeth shows great variability (e.g., Amardeep et al. 2014). It has become clear that teeth have complicated root canal systems rather than the simplified canals shown by Hess and Zurcher in 1925. Their study of mesiobuccal root canal systems of maxillary first and second molars became the basis of multiple and

more detailed investigations regarding the anatomic complexities of root canal systems (e.g., Carrotte 2004; Gulabivala 2014). The internal topography of root canals does not always demonstrate the simplicity of the external anatomy of the tooth. In some cases, the number of roots, their morphology and root canal systems can vary and change. The precise etiology of accessory canal formation remains elusive. Among the factors believed to contribute to canal configura-

tions are sex (Ahmed et al. 2007; Kottoor et al. 2013), geography (Sert and Bayirli 2004; Awawdeh et al. 2008; Al-Qudah and Awawdeh 2009) and ethnicity (Kottoor et al. 2013; Amardeep et al. 2014).

While it is evident that accurate knowledge of the number, the variability, and the morphology of root canal systems are essential during endodontic treatment (e.g., Plotino et al. 2013), what is less evident is that analyses of root canal systems can provide a vital source of information for anthropological research. Unfortunately, such studies are usually restricted to studies of contemporary populations (e.g., Ahmed et al. 2007; Torres et al. 2015). Meanwhile, the studies of the root canal systems in relation to historical populations are very rare and are only occasionally discussed (Prado-Simón et al. 2012; Ceperuelo et al. 2014; Ramirez-Salomón et al. 2014). Even less attention is given to the variability of root canal systems in paleoanthropological research (e.g., Harvati et al. 2003; Rosas et al. 2006). One of the reasons why researchers focus on contemporary dental material is the often poor preservation of archaeologically derived human remains due to diagenetic and taphonomic changes (e.g., White and Folkens 2005). Another problem arises from the need for researchers to have specialized radiographic device qualifications to study root canal systems.

Several different methods of root system classifications have been employed in clinical endodontic studies. However, in anthropological studies, this issue is often not addressed. Therefore, the aim of this paper is to review the literature concerning for the number of root canals, their morphology, and their methods of classification.

Studies of root canal system in each tooth identified through specialist databases plus hand searching were included. For relevant literature following databases were searched: Wiley Online Library, PubMed, Scopus, Science Direct, BioMed Central.

The literature was divided into anatomic studies and clinical case reports with primary interest to anatomic studies and morphology of root canal system occasionally supplemented by individual clinical cases described in the endodontic studies. Anatomic studies data were used for overview of internal morphology of the tooth. Studies in the field of endodontics allowed authors to depict the percentage distribution of the number of canals in each type of tooth.

Morphology of the root canal system

The anatomic root is covered by cementum and extends from the cement-enamel junction (CEJ) to the apex. A root canal starts as a funnel-shaped canal orifice within the pulp chamber, generally below the center of the cusp tips, and ends in an anatomical foramen, which opens onto the root surface between 0 and 3 mm from the center of the root apex. A vascular-nerve bunch enters the canal through the anatomical foramen (Carrotte 2004; Wright 2007; Gopikrishna 2010). The canals taper towards the apex, following the external outline of the root. The canals are ovoid in cross-section, having their maximum diameter at or just below the orifice. In a longitudinal section, the canals are broader buccolingually than on the mesiodistal plane (e.g., Carrotte 2004; Vertucci 2005). In some cases, a single tooth root manifests more than

Table 1. The morphological differentiation of teeth affects the diverse variability of root canals.

Tooth	No. of canals	Comments	References
Maxillary teeth			
Central incisor	1		Vertucci, 1984; Carrotte, 2004;
Lateral incisor	1	Flattened mesio-distally, the apical third tends to curve distally	Cleghorn et al. 2012
Canine	1	Flattened mesio-distally; oval shape	
	2		
1st premolar	2 (80% ¹ –95% ²)	2 canals in the apical third into the buccal root occur exceptionally	Vertucci, 1984; ¹ Pécora et al. 1991; ² Carrotte, 2004
2nd premolar	2 (24% ¹ –58% ²)	An additional canal occurs in 59% of cases	² Bellizi and Hartwell, 1985; ¹ Carrotte, 2004
	1 (40% ¹ –75% ²)		Vertucci, 1984; ¹ Bellizi and Hartwell, 1985; ² Carrotte, 2004
1st molar	3	C-shaped canal occurs in 0.12% of cases	Vertucci, 1984, 2005; Carrotte, 2004; Cleghorn et al. 2012
	4 (56%–93%)		Carrotte, 2004; Vertucci, 2005; Cleghorn et al. 2006; Fernandes et al. 2014
2nd molar	3 (63%)	C-shaped canal occurs	Vertucci, 1984; Carrotte, 2004; Cleghorn et al. 2012; Fernandes et al. 2014
	4 (37%)		
3rd molar	numerous	Complex root canal systems	Carrotte, 2004
Mandibular teeth			
Central incisor	1 (58%)		Vertucci, 1984; ¹ Carrotte, 2004; Cleghorn et al. 2012
	2 (25%–42% ¹)	Only in 1% of cases two separate foramina occur	
Lateral incisor	1 (58%)	Only in 1% of cases two separate foramina occur	
	2 (25%–42%)		
Canine	1	Flattened mesio-distally	
	2		

Tooth	No. of canals	Comments	References
1st premolar	1 (73%–89%)	One canal may separate into two; The C-shaped canal occurs in 10–14% of cases	
	2 (25%–27%)	2 or more canals in women	Baisden et al. 1992; Sikri and Sikri, 1994; Carrotte, 2004; Jafarzadeh, 2007; Kottoor et al. 2013; Fernandes et al. 2014
2nd premolar	1 (62%–85%)	1 canal may separate into two; C-shaped canal occurs Multiple canals in men	Vertucci, 1984; Carrotte, 2004
	2 (5%–15%)		Carrotte, 2004; Kottoor et al. 2013; Fernandes et al. 2014
1st molar	3 (67%)	2 canals in the mesial root and 1 canal in the distal root occurs; C-shaped canal occurs; An additional canal occurs in distal root	Vertucci, 1984, 2005; Carrotte, 2004; Cleghorn et al. 2006; Jafarzadeh and Wu, 2007; Cleghorn et al. 2012
	4 (25%–29% ¹ , 33% ²)		¹ Skidmore and Bjorndal, 1971; ² Cattotte, 2004
2nd molar	3 (79%)	2 canals in the mesial root and 1–2 canals in the distal root occur C-shaped canal occurs	Vertucci, 1984; Carrotte, 2004; Jafarzadeh and Wu, 2007; Cleghorn et al. 2012; Fernandes et al. 2014
	4 (7–42%)	An additional canal occurs in distal root	Cleghorn et al. 2012
3rd molar	numerous	Irregular canal configurations; C-shaped canal occurs	Carrotte, 2004; Fernandes et al. 2014

a single canal. Frequently, apart from the main canal root, shorter accessory and lateral canals, smaller in diameter, are present, marking the spread from the pulp chamber to the periodontium (Dakokar et al. 2015). A branch of the main pulp canal or chamber, referred to as an accessory canal, communicates with the external surface of the root. A lateral canal, generally extending horizontally from the main root canal, is placed in the coronal, or middle third, of the root (Vertucci 2005).

One of the most essential anatomic variations of the root canal system is the

C-shaped canal configuration, first documented by Cooke and Cox in 1979. It is named for the C-shaped cross-sectional morphology of the root and root canal (Jafarzadeh and Wu 2007; Fernandes et al. 2014). The main cause of a C-shaped canal inside the C-shaped root is the failure of Hertwig's epithelial root sheath to fuse to the lingual or buccal side (Gulabivala et al. 2002; Fan et al. 2004a; Jafarzadeh and Wu 2007). A basic anatomic feature that makes the canal cross-sectional and 3D shape variable along the C-shaped root is the presence of a fin or web connecting the mesial and distal in-

dividual canals (Fan et al. 2007). However, a C-shaped canal with a single swath of canal occurs rarely. Two or three canals may be found throughout the length of the root in the case of an uninterrupted C-shape (Al-Fouzan 2002; Fan et al. 2004a; Jafarzadeh and Wu 2007). Such C-shaped canals occur with a frequency of 2.7% to 8%, and may be present in teeth with fused roots when a deep groove is present on the lingual or buccal surfaces of the root. They are mostly observed in mandibular second molars. The C-shaped canal can also appear in other maxillary and mandibular molars and premolars, even in maxillary lateral incisors (Fan et al. 2004a,b 2007; Jafarzadeh and Wu 2007). Researchers have revealed that there are links between radiographic features of mandibular second molars with a C-shaped canal system and the canal anatomy by comparing radiographic features with microcomputed tomography (μ CT) scanned canal images. However, the complicated morphology of the posterior teeth and the effect of image superimposition of the surrounding hard tissue can make identification of the C-shaped canal system anatomy in mandibular second molars difficult (Fan et al. 2008a,b). In some cases, differentiating between C-shaped canals and canals in which single or three canals join apically may be tough (Al-Fouzan 2002). The main number of root canals and their morphological variations in each tooth are shown in Table 1.

The above table shows only the primary and most common number of canals reported in the literature; however, the variability of the root canal system differs widely. Depending on the research method and sample, the percentage of additional canals may vary.

Different classifications of root canal configurations

The canals in roots may reveal many variations in their configuration. According to this, various researchers proposed numerous classification systems based on the number and morphology of root canals in each type of tooth, depending on the teeth used in research and group from which these teeth were obtained. It appears, that root canal morphology can vary between anterior and posterior teeth. There is a lack of unity in the multiplicity of existing classification systems. Comprehensive classifications may help in truly managing root canals and their morphological complexities. A classification of complex canal systems has been compiled, with additional canal configurations described below. In general the canals start from the pulp chamber, then runs straight to the apex; alternatively they may divide or join prior to exiting the apical foramina (as shown in Fig. 1).

A classification system based upon mandibular central incisors, lateral incisors and canines was offered by Vertucci (1974) who classified root canals into four types. Type I (1) a single canal is present from the pulp chamber to the apex, type II (2-1), type III (1-2-1) and type IV (2) explained as above.

In a subsequent study Vertucci (1984), using human maxillary and mandibular permanent teeth (except for third molars), identified eight root canal configurations, of which the first four types were identical to those provided in his 1974 classification scheme (Vertucci 1974). The remaining types are summarized as follows. Type V (1-2) one canal leaves the pulp chamber and divides just before the apex into two separate and distinct canals with separate apical fo-






















































Vertucci (1974)	Vertucci (1984)	Gulabivala et al. (2001)	Gulabivala et al. (2002)	Sert, Bayirli (2004)	Peiris (2007)	Al-Qudah, Awawdeh (2009)	Sert et al. (2011)	
								
Type I	Type I (1)	Type (3-1)	Type (3-4)	Type (1-2-3-2-1-3)	Type (1-2-3-2-1)	Type (2-3)	Type (2-1-2-1)	Type (3-1-2)
								
Type II	Type II (2-1)	Type (2-1-2-1)	Type (2-1-2-1)	Type (1-3-4-1)	Type (1-2-3-2)	Type (1-2-3)	Type (2-3)	Type (3-1-2-1-2)
								
Type III	Type III (1-2-1)	Type (4-2)	Type (3-1)	Type (3-2-1)	Type (2-3-2)	Type (3-1-2)	Type (2-3-1)	Type (2-3-4-2)
								
Type IV	Type IV (2)	Type (3-2)	Type (3-2)	Type (2-3-2-1-2)	Type (2-1/2-1)		Type (2-3-2)	Type (2-5-1)
								
Type V (1-2)		Type (2-3)	Type (2-3)	Type (1-2-4-2)	Type (2-3-1-3-1-4)		Type (3-1)	
								
Type VI (2-1-2)		Type (4)		Type (1-3-1-2)	Type (3-2-1)		Type (3-2)	
								
Type VII (1-2-1-2)		Type (5-4)		Type (1-2-4-3-1)			Type (3-2-1)	
								
Type VIII (3)				Type (1-2-3-1)			Type (3-2-3)	

Fig. 1. Root canal configurations in human permanent teeth

ramina, type VI (2-1-2), type VII (1-2-1-2), type VIII (3).

In more recent years, Peiris (2007) identified three additional canal configurations among SriLankan and Japanese individuals, based on Vertucci's revised 1984 scheme and the same types of teeth. Type 2-3 two canals leave the pulp chamber, then one divides into two, resulting in three canals exiting the apical foramina, type 1-2-3, type 3-1-2.

Mandibular first and second molars in a Jordanian sample provided the basis for the classification system developed by Al-Qudah and Awawdeh (2009). Additional modifications not included in the Vertucci classification (1984) were described. These include: type 2-1-2-1: in which two separate canals leave the pulp chamber, merge at the mid-point, divide again into two separate canals, and then join to exit as a single canal, as well as types 2-3, 2-3-1, 2-3-2, 3-1, 3-2, 3-2-1, and 3-2-3.

The two root canal classification systems developed by Gulabivala et al. (2001, 2002) were based on observations of root canal configurations among the mandibular molars in a sample of Burmese and Thai individuals, respectively. Additional types not present in the Vertucci (1984) classification were found. In the first case seven canal systems were described and these include: type 3-1: three canals joining into one; type 2-1-2-1, type 4-2, type 3-2, type 2-3, type 4, type 5-4. In the second sample five canal configurations were defined and these include: type 3-4: three canals leave the pulp chamber, and then one divides into two, the other two run straight to the apex, finally resulting in four canals exiting the apical foramina, type 2-1-2-1, type 3-1, type 3-2, type 2-3.

Sert et al. (2011) encountered four new root canal configurations in their study of maxillary and mandibular per-

manent molars belonging to members of a Turkish sample, not included in the other classification schemes (e.g., Vertucci 1984; Gulabivala et al. 2001, 2002). The first three occur in the mandibular first molar, while the fourth occurs in the type mandibular third molar. Type 3-1-2 three canals leave the pulp chamber, merge in the mid-root region and become a single canal, they continue as buccal and lingual canals and ends as two foramina, type 3-1-2-1-2, type 2-3-4-2, type 2-5-1.

The classification systems described above were intended to be applied to members of both sexes. A different approach to root canal classification has been offered by Sert and Bayirli (2004), who proposed a classification system differentiated by sex. These studies were conducted on all of the mandibular and maxillary permanent teeth, with the exception of third molar, among Turkish individuals. Fourteen new root canal configurations not included in the other classification systems (e.g., Vertucci 1984; Gulabivala et al. 2001, 2002) were provided. Among males, they found four types: 1-2-3-2-1-3; 1-3-4-1; 3-2-1; 2-3-2-1-2. The first type (1-2-3-2-1-3) belongs to the male mandibular central incisor: one wide root canal leaves the pulp chamber, separates into two (labial and lingual) between the cervical and middle thirds of the roots; then, the labial canal separates into two in the middle third portion of the root. These two branches join again in the proximity of the apex, and then diverge into three canals. The second type (1-3-4-1) belongs to the male mandibular canine, the third (3-2-1) and fourth type (2-3-2-1-2) pertains to the male maxillary first molar. They found greater variability in root canal morphology among females. They proposed ten different types: 1-2-4-2; 1-3-1-

2; 1-2-4-3-1; 1-2-3-1; 1-2-3-2-1; 1-2-3-2; 2-3-2; 2-1/2-1; 2-3-1-3-1-4; 3-2-1, from which types 1-2-4-2 (a single, wide canal leaves the pulp chamber and splits into two branches at the cervical portion. The labial canal splits into three branches at the end of the middle third of the root, and then the three canals at the labial side join at the apical one-third and end as two separate foramina); 1-3-1-2; 1-2-4-3-1; 1-2-3-1; 1-2-3-2-1 and 1-2-3-2 belongs to the female mandibular central incisors, types 2-3-2; 2-1/2-1 pertains to the female mandibular lateral incisor, type 2-3-1-3-1-4 belongs to the female maxillary second molar, type 3-2-1 belongs to the female single-rooted maxillary second molar.

Classification of C-shaped canal configurations

The numerous classifications of C-shaped canals were proposed to better understand the root canal anatomy of these types of configurations. Classifications are shown in Figure 2.

The primary classification was proposed by Melton et al. (1991) based on the two evaluation methods, light microscopy and stereomicroscope. The three arbitrary levels of the root were checked in terms of changing canal shape throughout its length. Accordingly, the clinical crown morphology and the appearance of the canal orifice may not render the actual canal anatomy. The proposed classifications were compiled into three categories. The most common is the second type of the C-shaped canal (Melton et al. 1991; Jafarzadeh and Wu 2007). These three categories are presented as: category I – the C-shaped outline is defined by a continuous C-shaped

canal running from the pulp chamber to the apex without any separation; category II – the semicolon-shaped orifice in which dentine separates a main C-shaped canal from a buccal or lingual C-shaped canal in the same section; category III – there are two or more separate, individual canals. So, other classifications were then proposed. Fan et al. (2004a) was modified this method according to the anatomic and radiographic features in root canals. He mentioned that the majority of teeth with C-shaped canal systems showed an orifice with a continuous “C” configuration. The apical portion was divided in 32% of the canals, most of which did so within 2 mm from the apex.

In the anatomic classification, a tooth was defined as having a C-shaped canal system when one or more cross-sections assumed a C1, C2, or C3 configuration. The level of the CEJ was taken where the enamel occupied one-half of the perimeter of that cross-section. The orifice of the root canal was taken at the level where the floor of the pulp chamber became discernible. Teeth with only a single oval or round canal (i.e., C4) from the crown to the apex were not classified as having a C-shaped canal, which should be reflected as a variation when other parts of the canal showed the “C” configuration. Fan et al. (2004a) described all mandibular second molars as having a C-shaped canal system when teeth revealed three features: fused roots, a longitudinal groove on the lingual or buccal surface of the root, and one cross-section of the canal belonging to the C1, C2, or C3 configuration (Fan et al. 2004a). Proposed categories were as follows: category I (C1): the shape is an interrupted “C” with no separation or division; category II (C2): the canal shape resembles a semicolon resulting from the discontin-

uation of the "C" outline, but either angle α or β are no less than 60° (as shown in Figure 2, angle β is more than 60°); category III (C3): two or three separate canals and both angles α and β are less

than 60° (as shown in Fig. 2, both angles α and β are less than 60°); category IV (C4): only one round or oval canal in that cross-section; category V (C5): no canal lumen is observed (which is usually seen

Melton et al. (1991)	Anatomic	Fan et al. (2004a,b)	Radiographic
		Measurement of angles	
Category I (C1)	Category I (C1)		Type I
Category II (C2)	Category II (C2)		Type II
Category III (C3)	Category III (C3)		Type III
	Category IV (C4)		
	Category V (C5)		

Fig. 2. The C-shaped root canal classifications. A and B-ends of one canal cross-section; C and D-ends on the other canal cross-section; M-middle point of line AD; α -angle between line AM and line BM; β -angle between line CM and line DM

near the apex only) (Fan et al. 2004a; Jafarzadeh and Wu 2007; Kirilova and Topalova-Pirinska 2014).

In the radiographic classification, the teeth were scanned at 0.5 mm interval thickness via μ CT and observed at 11 levels; C-shaped roots were classified into three types according to their radiographic features. Type I: conical or square root with a vague, radiolucent longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal that merged into one before exiting at the apical foramen/foramina. Type II: conical or square root with a vague, radiolucent, longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal, and the two canals appeared to continue on their own pathway to the apex. Type III: conical or square root with a vague, radiolucent longitudinal line separating the root into distal and mesial parts. There was a mesial and a distal canal: one canal curved toward and superimposed this radiolucent line when running towards the apex, and the other canal appeared to continue on its own pathway to the apex (Fan et al. 2004b; Jafarzadeh and Wu 2007).

The above descriptions proposed by an array of researchers highlights the diversity and lack of homogeneity across the numerous classification systems. From the perspective of anthropological research it poses difficulties. Due to the uniqueness of bioarchaeological material, future research should focus on proposing a clear unequivocal classification system.

Methods of root canal system detection

Standard intraoral radiography is an extensively used, reliable, clinical method

of determining canal anatomy; nevertheless, it is characterized by a number of disadvantages and problems that complicate radiographic assessment. The first inconvenience is exposure to ionizing radiation, especially when more than one view is needed. Furthermore, this is a time-consuming process, especially when more than one view is needed. The second inconvenience relates to differences in brightness, contrast and acquisition geometry between radiographs (Fan et al. 2008a). Standard radiography can provide only two-dimensional images for three-dimensional objects and can only show main canals along the root, with a low probability of showing the extent of the complexities of root canal systems, for additional root canals can be missed on the radiograph (Al Nazhan 1999). To visualize the buccolingual anatomy with greater accuracy, two preoperative radiographs are made: one with a 90° angulation to the tooth in a buccolingual direction and a second at a mesial angle of approximately 20° (Seo and Park 2004).

The direct digital radiography (DDR) system is now in operation clinically and, as an addition to alternative radiographic documentation, offers more benefits than conventional radiography. The DDR system is easy and fast to use, thereby exposing the patient to less radiation, reducing the time between exposure and image interpretation, offering the ability to handle the captured image digitally. However, this technique has two basic demands: contrast variation in serial target images and strict repetition of serial image positions (Fan et al. 2008b).

Radiovisiography is a type of DDR with many potential advantages and disadvantages, namely background noise, such as in tooth and bone tissue images. Background noise from normal anatomic

structures can be reduced using a better and more reliable method – digital subtraction radiography (DSR) – which excludes identical image regions in a series of radiographs received in the same exposure position at different time intervals and provides two-dimensional images that reflect the canal anatomy with high sensitivity and specificity. The application of DSR in observing the root canal anatomy has not been examined completely until now. This method can establish the presence of anatomic changes by enhancing the accuracy of continuous canal recognition of a C-shaped canal system in mandibular second molars in the apical area, emphasizing the details of canal anatomy, verifying the shape of the canal anatomy preparation, and highlighting the canal image against other tissue images. When using DSR, it is essential to use a contrast medium, such as 76% compound meglumine diatrizoate (Fan et al. 2007, 2008b).

A water-soluble radiographic contrast medium can be introduced into the root canal system by injecting the medium into the canal with a syringe or placing the teeth under a vacuum container using a suction device to pull the medium into the canal irregularities. This technique has also been used by clinicians to establish the capability to clean and shape the root canal system. However, using a water-soluble radiographic contrast medium is not a new idea. When using image superimposition, as the area has thick cortical bone plates, such as the area surrounding the mandibular second molars where the buccal and lingual cortical bone plates are much thicker than other areas, it is difficult to describe the morphology of the root canal system, in particular, when interpreting the C-shaped canal system in the mandib-

ular second molars. Even when a bone superimposition exists, introducing the contrast medium into a C-shaped canal system in the mandibular second molar provides more information about the canal anatomy, especially in the apical area (Fan et al. 2007, 2008a,b). However, these techniques could be used in a laboratory study only, as they are inefficient when distributing a contrast medium in root canals. DSR is used to reveal the anatomic features of C-shaped canal systems in mandibular second molars with the aid of an intraradicular contrast medium (Fan et al. 2008a,b).

The μ CT scans are used to establish details in root canal anatomy and morphologic changes in cross-sections of teeth non-destructively. This technique creates a three-dimensional reconstruction of the internal and external anatomy of the tooth. The images can be examined using different representations as a multiplanar reformation and three-dimensional surface rendering. Without superimposition, the anatomic structures can be rotated on any spatial plane. μ CT scans have been successfully used to study root canals qualitatively and quantitatively in-vivo. The scanning interval is usually 0.5 mm (e.g., Chirani et al. 2007; Michetti et al. 2010).

Cone-beam computed tomography (CBCT), also known as Dental Volumetric Tomography, is a non-invasive method that does not destroy the sample. This method has become a popular tool for investigating root canal anatomy, because it enables visualization of fine details in three-dimensional imaging without visual noise, and it also allows the overlapping visualization of adjacent structures. As such, CBCT represents a very useful method for the assessment of internal root canal morphology in well-preserved

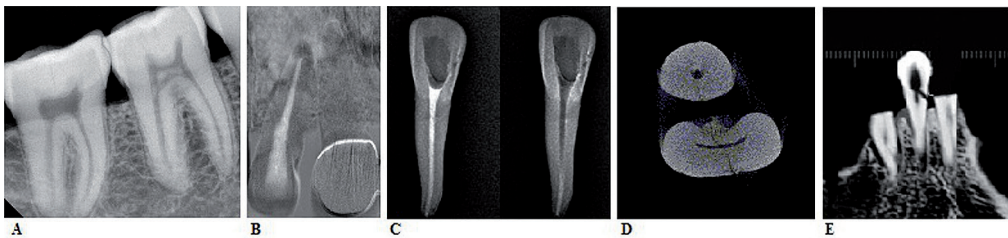


Fig. 3. An examples of using different visualization methods of root canal systems. A: Direct Digital Radiography; B: Digital Subtraction Radiography. It reverses the shadows of gray for diagnostic purpose; C: Digital Subtraction Radiography with contrast medium; D: Microtomography; E: Cone-beam computed tomography

teeth recovered from archeological context because it allows precise evidence to be received without requiring the tooth to be extracted for analysis (e.g., Cepeluelo et al. 2014; Torres et al. 2015). Using CBCT, it is possible to detect periapical pathologies faster than with conventional radiographs. CBCT images, derived from sagittal, frontal and axial plane images, eliminates the overlap of anatomical structures in images (e.g., it allows for the analysis of the roots of posterior upper teeth and their apex region without casting a shadow on the zygomatic bone, alveolar and other roots) (Arslan et al. 2015; Torres et al. 2015). CBCT technology uses isotropic voxels, empowering exact linear geometric and three-dimensional measurements of the acquired data (equal dimensions in all three planes of space) (Torres et al. 2015). The following cross-sections are obtained: contact points, transsectal, axial, frontal and sagittal. CBCT currently represents the “gold standard” in identifying root canal anatomy and has been progressively used over the last year for in-depth studies of the internal morphology of the root. This is because the exact modified canal staining and clearing technique is more precise in diagnosing apical radiolucencies than digital x-rays (e.g., Benyó 2012; Amardeep et al. 2014). To demonstrate an apparently frequent

occurrence as uncommon, a rare form of the number of roots may need to be adjusted before the operative radiographic technique (Loh 1998). The differences between the visualizations methods are shown in Figure 3.

Ethnic and geographic variations on root canal morphology

Observed variability of root canal systems may be associated with study design (*in vitro* or *in vivo*), technique of canal identification or ethnic group. Unfortunately, research conducted on the complexity of root canal systems occurs only in the field of endodonty (e.g., Sert and Bayirli 2004; Al-Qudah and Awawdeh 2009; Amardeep et al. 2014). Consequently, researchers using various methodologies describe characteristic types of canals noted in each type of tooth. The ethnic background of the sample considered is often not take into consideration as a criterion for inclusion in many studies. A number of researchers have pointed out that the number and types of root canals may vary widely in specific parts of the world (e.g., Walker 1988a; Melton et al. 1991, Haddad et al. 1999). Meanwhile, it seems that in some parts of the globe, there is a tendency to emerge spe-

cific pattern of root canal configurations in terms of space and time, despite the using various methods of inner anatomy of the tooth visualization (e.g., Dahlberg 1965; Valencia de Pablo et al. 2010). One of these is variations of pulp chamber anatomy, especially in terms of C-shaped canal systems. Such C-shaped canals strongly differentiate and typify ethnic groups from specific parts of the globe, especially Asian individuals, who have a much higher prevalence of C-shaped canals than do members of other populations (Jafarzadeh and Wu 2007; Kirilova and Topalova-Pirinska 2014; Ramírez-Salomón et al. 2014).

As we know, the studies of genetic information can reveal a reconstruction of human population's history (e.g., Abu-Amero et al. 2007; Gorostiza et al. 2012). However, these analyses are not always possible on the bioarchaeological material, due to the diagenetic changes that occur to bones and teeth after the death of an individual (e.g., Gorostiza et al. 2012; Ramírez-Salomón et al. 2014). Therefore, discerning analysis inner anatomy of root in terms of canal systems may be helpful in this respect. We can assume that the knowledge of the diversity of root canals in the past populations may provide important information relating to the direction of migration and genetic relationship. However a small number of research discuss this aspect (e.g., Abu-Amero et al. 2007; Summerer et al. 2014; Al-Saud et al. 2015).

It seems that, due to the variability of root canal systems, the current population of Myanmar reflects gene flow between populations with Indian and Chinese origins (Donnison 1970). The majority of the inhabitants of Myanmar (70%) possess Mongoloid traits, but the rest of the population is composed of mi-

nority groups who inhabit the surrounding mountains (e.g., Donnison 1970; Gulabivala et al. 2001). In this respect, the endodontic research was confirmed by mitochondrial DNA analysis of Myanmar (Burma) (Summerer et al. 2014). Myanmar haplogroup distribution showed a typical Southeast Asian pattern, but also displayed quite a few parallels to Northeast Asian and Indian individuals. The mitochondrial diversity of Southeast Asians represented by the population of Myanmar could be explained by long population history and geographical position on the trade route between the giant empires of India and China (Summerer et al. 2014).

Gulabivala et al. (2002), examining root and canal morphology among the molars of their Thai sample observed that these individuals exhibited similarities and departures from the morphologies observed among the average Caucasian and Chinese traits. The current population of Thailand is believed to have originated from a mixture of Indian and Chinese emigrants. The testing of mitochondrial DNA of living people in China and some countries in Southeast Asia resulted in a phylogenetic tree that links ancient skeletons and members of the living population. As the results indicate that migration may have taken place about 3.000 years ago. Thai ancestors may have shared the same ancestors from people in China and Southeast Asia (JimSEA 2006).

The frequency (10.6%) of C-shaped root canal configurations in Saudi Arabia populations lies between 2.7% reported earlier and 31.5 % occurrence of this trait characteristic for Chinese described by many researchers (e.g., Yang et al. 1988). The geographic position of Saudi Arabia in particular, and the Middle East in general, may provide an expla-

Table 2. A summary of the variability in root systems in contemporary populations.

Geographical region	Teeth	No. of canal	Comments	References
Europe: Belgian population	Mandibular molars	3	2 canals in mesial root: type V, I, III (Vertucci) and 1 canal in distal root occurs: type I (Vertucci); C-shaped canal occurs in 10% of cases	Torres et al. 2015
Asia: Korean, Thai, Burmese, Indian populations	Majority of teeth		C-shaped canal configuration: category II (Melton)	Seo and Park, 2004
	Canine	1	Type I (Vertucci)	Amardeep et al. 2014
	Mandibular first molar	2–4	2 distal canals occur;	Gulabivala et al. 2001
	Mandibular second molar	3	2 mesial and 1 distal canal occurs: type IV, I (Vertucci); C-shaped canal occurs in 7.5–10% of cases	Gulabivala et al. 2002
Europe/West Asia: Caucasian, Turkish populations	Maxillary canine	1	Type I (Vertucci)	Amardeep et al. 2014;
		1	1-3-4-1 (Sert and Bayirli)	Sert and Bayirli, 2004; Amardeep et al. 2014
	Mandibular first and second molars	3	Type I (Vertucci)	Gulabivala et al. 2001;
	Mandibular third molar	2–3 or more		Gulabivala et al. 2001;
Middle East: Iranian, Lebanese, Jordanian, Saudi Arabian populations	Maxillary canine	more than 1		Amardeep et al. 2014;
	Mandibular first molar	3–4		Al-Nazhan, 1999 Al-Qudah and Awawdeh, 2009
	Mandibular second molar	2–4	C-shaped canal occurs in 10.6– 19.1% of cases	Al-Nazhan, 1999 Al-Qudah and Awawdeh, 2009 Haddad et al. 1999 Al-Fouzan, 2002
Far East: Japanese, Hong Kong Chinese populations	Mandibular first molar	3	2 canals in mesial and 1 canal in distal root occurs; 2–3 canals occur in distal root exceptionally;	Walker, 1985, 1988a; Al-Nazhan, 1999
	Mandibular second molar		C-shaped canal occurs in 14–52% of cases, but in 70% of cases occur in the contralateral teeth	Gulabivala et al. 2002; Walker, 1988b; Manning, 1990; Jafarzadeh and Wu, 2007; Kirilova and Topalova-Pirinska, 2014; Gao et al. 2006

Geographical region	Teeth	No. of canal	Comments	References
Africa: Sudanese population	Mandibular first molar	4	2 canals in the mesial and 2 canals in the distal root occur: type IV, II (Vertucci);	Ahmed et al. 2007
	Mandibular second molar	3	2 canals in mesial root occur; 1 canal in distal root occurs: type IV, II (Vertucci); C-shaped canal occurs in 10% of cases	Ahmed et al. 2007
North America: East Greenland Eskimo population	Maxillary first premolar	2		Pedersen, 1949
Central America: Yucatan population	Mandibular second molar		C-shaped canals occur with the second highest frequency of appearance worldwide	Ramirez-Salomón et al. 2014
South America: Chilean population	Mandibular molar	3	2 canals in mesial root occur: type III, IV, V (Vertucci); 1 canal in distal root occurs: type I (Vertucci); C-shaped canal occurs in 10% of cases	Torres et al. 2015

nation of this phenomenon (Al-Fouzan 2002). The other explanation is given by a mitochondrial DNA analysis of Saudi Arabian group that reveals contributions from Africa and the Near East almost solely (Abu-Amro et al. 2007). The genetic analysis was supported by an analysis conducted by Al-Saud et al. (2015), which presented genetic stratification within the Saudi Arabia population. The similarity of Saudi Arabians with Central-East Asian, European and Qatari populations was identified.

The ethnic ancestry of the Native Americans are associated with populations that originated from Northeast Asia during two or three migratory waves, by crossing the Bering Strait (e.g., Turner 1990; Cavalli-Sforza et al. 1994). Genetic admixture between members of native groups and European and to a lesser ex-

tent the Africans that has occurred over the course of the last five centuries accounts for the appearance of modern Mesoamerican and Yucatean populations. The 35% frequency of C-shaped root canal configurations found among of Yucateans places this population as the second highest incidence of C-shaped canals worldwide. These results provide new and strong evidence that strengthens support for the settlement of the Americas by ancient populations from Northeastern Asia (Ramírez-Salomón et al. 2014). The frequency of the C-shaped root canal configurations found among mandibular second molars in Lebanese sample (19.1 %) lies between the 31.5% frequency reported by Yang et al. (1988) and the 8 % frequency reported by Cooke and Cox (1979). This phenomenon may be explained by the geographic position

of Lebanon in particular and the Middle East in general (approximately half-way between the Far East and North America) (Haddad et al. 1999). General variability in root systems are shown in Table 2.

The above examples show the possible use of research on the variability of root canals in historical populations. May be the deficiency of genetic material from the past population, can be replaced by exact analyses of root system.

Conclusions

The comprehensive analyses of root canal systems are mainly using in endodontics. This topic is also applicable to anthropological research among members of contemporary populations. However, the study of bioarchaeological material has many limitations. In such material, the external and internal structures are often poorly preserved, for the roots are often damaged, which prevents more detailed analysis. These factors often preclude the use of some methods of visualization of root canal complexities; for example, the water-soluble radiographic contrast medium cannot be introduced into the root canal system due to the uniqueness and significance of some material to later research. The contamination of the dry material fundamentally prevents the success of genetic research, consequently the research of root canal systems may be helpful in tracing the ethnic origin of the populations. Therefore, in the study of dental samples of historical material, the simplest, most basic and unified methods for detection and classification of the root canal systems are needed.

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Authors' contributions

AP collected the literature and wrote the manuscript. JT conceived of the paper aim and design and revised the manuscript. Both authors read and approved the final manuscript.

Conflict of interest

The Authors declare that there is no conflict of interests.

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References

- Abu-Amero KK, González AM, Larruga JM, Bosley TM, Cabrera VM. 2007. Eurasian and African mitochondrial DNA influences in the Saudi Arabian population. *BMC Evol Biol* 7: 32.
- Ahmed HA, Abu-bakr NH, Yahia NA, Ibrahim YE. 2007. Root and canal morphology of permanent mandibular molars in a Sudanese population. *Int Endod J* 40:766–771.
- Al-Fouzan KS. 2002. C-shaped root canals in mandibular second molars in a Saudi Arabian population. *Int Endod J* 35:499–504.
- Al-Nazhan S. 1999. Incidence of four canals in root-canal treated mandibular first molars in a Saudi Arabian subpopulation. *Int Endod J* 32:49–52.
- Al-Saud H, Walkil SM, Mayer BF, Falchi M, Dzimir N. 2015. The genetic structure of

- the Saudi Arabian population. Available at: www.racehist.blogspot.com.
- Al-Qudah AA, Awawdeh LA. 2009. Root and canal morphology of mandibular first and second molar teeth in a Jordanian population. *Int Endod J* 42:775-784.
- Amardeep NS, Raghu S, Natanasabapathy V. 2014. Root canal morphology of permanent maxillary and mandibular canines in Indian population using cone beam computed tomography. *Anat Res Int*. doi: 10.1155/2014/731859.
- Arslan H, Capar ID, Ertas ET, Ertas H, Ackay M. 2015. A cone-beam computed tomographic study of root canal systems in mandibular premolars in a Turkish population: Theoretical model for determining orifice shape. *Eur J Dent* 9:11-19.
- Awawdeh L, Abdullah H, Al-Qudah A. 2008. Root form and canal morphology of Jordanian maxillary first premolars. *J Endod* 34:956-961.
- Baisden MK, Kulid JC, Weller RN. 1992. Root canal configuration of the mandibular first premolar. *J Endod* 18:505-508.
- Bellizzi R, Hartwell G. 1985. Radiographic evaluation of root canal anatomy of in vivo endodontically treated maxillary premolars. *J Endod* 11:37-39.
- Benyó B. 2012. Identification of dental root canals and their medial line from micro-CT and cone-beam CT records. *Biomed Eng Online* 11:81.
- Carrotte P. 2004. Endodontics: Part 4. Morphology of the root canal system. *Br Dent J* 197:379-383.
- Cavalli-Sforza LL, Menozzi P, Piazza A. 1994. The history and geography of human genes 1st edn. Princeton, NJ, USA: Princeton University Press.
- Ceperuelo D, Lozano M, Duran-Sindreu F, Mercade M. 2014. Root canal morphology of Chalcolithic and Early Bronze Age human populations of El Mirador Cave (Sierra de Atapuerca, Spain). *Anat Rec* 297:2342-2348.
- Chirani RA, Jacq JJ, Chevalier V., Calas P. 2007. Microtomography: a method to assess root canal geometry and changes after endodontic preparation. *Eur Cell Mater* 13.
- Cleghorn BM, Boorberg NB, Christie WH. 2012. Primary human teeth and their root canal systems. *Endod Topics* 23:6-33.
- Cleghorn BM, Christie WH, Dong CC. 2006. Root and root canal morphology of the human permanent maxillary first molar: a literature review. *J Endod* 32:813-821.
- Cooke HG, Cox FL. 1979. C-shaped canal configurations in mandibular molars. *J Am Dent Assoc* 99:836-839.
- Dahlberg A. 1965. Geographic distribution and origin of dentitions. *Int Dent J* 15: 348-355.
- Daokar SG, Kalekar (Yadao) AS, Ghunawat DB, Kakde DD. 2015. All the mandibular incisors with double canals in a single patient: a rare case. *J Int Oral Health* 7(2):1-4.
- Donnison FSV. 1970. Burma. London, UK: Ernest Benn Limited 32-34.
- Fan B, Cheung GSP, Fan M, Gutmann JL, Bian Z. 2004a. C-shaped canal system in mandibular second molars: Part I – Anatomical Features. *J Endod* 30:899-903.
- Fan B, Cheung GSP, Fan M, Gutmann JL, Bian Z. 2004b. C-shaped canal system in mandibular second molars: Part II – Radiographic Features. *J Endod* 30:904-908.
- Fan W, Fan B, Gutmann JL, Cheung GSP. 2007. Identification of C-shaped canal in mandibular second molars. Part I: Radiographic and anatomical features revealed by intraradicular contrast medium. *J Endod* 33:806-810.
- Fan B, Gao Y, Fan W, Gutmann JL. 2008a. Identification of a C-shaped canal system in mandibular second molars: Part II – The Effect of bone image superimposition and intraradicular contrast medium on radiograph interpretation. *J Endod* 34:160-165.
- Fan W, Fan B, Gutman JL, Fan M. 2008b. Identification of a C-shaped canal system in mandibular second molars: Part III – Anatomic features revealed by digital subtraction radiography. *J Endod* 34:1187-1190.

- Fernandes M, de Ataíde I, Wagle R. 2014. C-shaped root canal configuration: A review of literature. *J Conserv Dent* 17(4):312–319.
- Gao Y, Fan B, Cheung GSP, Gutmann JL, Fan M. 2006. C-shaped canal system in mandibular second molars. Part IV: 3-D Morphological analysis and transverse measurement. *J Endod* 32:1062–1065.
- Gopikrishna V. 2010. Root canal morphology and access preparation. In: Kohli A, editor. *Textbook of Endodontics*. India: Elsevier. p. 125.
- Gorostiza A, Acunha-Alonzo V, Regalado-Liu L, Tirado S, Granados J, Sámano D, Rangel-Villalobos H, González-Martin A. 2012. Reconstructing the History of Mesoamerican Populations through the Study of the Mitochondrial DNA Control Region. *PLoS ONE* 7: e44666.
- Gulabivala K, Aung TH, Alavi A., Ng Y-L. 2001. Root and canal morphology of Burmese mandibular molars. *Int Endod J* 34:359–370.
- Gulabivala K, Opasanon A, Ng YL, Alavi A. 2002. Root and canal morphology of Thai mandibular molars. *Int Endod J* 35:56–62.
- Gulabivala K, Ng YL. 2014. Tooth organogenesis, morphology and physiology. In: Gulabivala K, Ng YL, editors. *Endodontics*. Available at: www.pocketdentistry.com.
- Haddad GY, Nehme WB, Ounsi HF. 1999. Diagnosis, classification, and frequency of C-Shaped canals in mandibular second molars in the Lebanese population. *J Endod* 25:268–271.
- Harvati K, Panagopoulou E, Karkanis P. 2003. First Neanderthal remains from Greece: the evidence from Lakonis. *J Hum Evol* 45:465–473.
- Hess W, Dolamore WH, Zürcher E. 1925. *Anatomy of root canals of the teeth of the permanent dentition*. New York: William Wood & Co.
- Jafarzadeh H, Wu Y. 2007. The C-shaped root canal configuration: a review. *J Endod* 33:517–523.
- JimSEA. 2006. Bones tell story of Thai origin. Bangkok Post. Available at: www.freerepublic.com/focus/chat/1732491/posts.
- Kirilova J, Topalova-Pirinska S. 2014. C-shaped configuration of the root canal system-problems and solutions. *J of IMAB* 20:504–509.
- Kottoor J, Albuquerque D, Velmurugan N, Kuruvilla J. 2013. Root anatomy and root canal configuration of human permanent mandibular premolars: a systematic review. *Anat Res Int*. doi. 10.1155/2013/254250.
- Loh HS. 1998. Root morphology of the maxillary first premolar in Singaporeans. *Aust Dent J* 43(6):399–402.
- Manning SA. 1990. Root canal anatomy of mandibular second molars. Part II. C-shaped canals. *Int Endod J* 23:40–45.
- Melton DC, Krell KV, Fuller MW. 1991. Anatomical and histological features of C-shaped canals in mandibular second molars. *J Endod* 17:384–388.
- Michetti J, Maret D, Mallet JP, Diemer E. 2010. Validation of cone beam computed tomography as a tool to explore root canal anatomy. *J Endod* 36:1187–1190.
- Pécora JD, Saquy PC, Sousa Neto MD, Woelfel JB. 1991. Root form and canal anatomy of maxillary first premolars. *Braz Dent J* 2:87–94.
- Pedersen PO. 1949. *The East Greenland Eskimo dentition, numerical variations and anatomy*. Copenhagen: CA Reitzels Forlag 129:158–161.
- Peiris R. 2007. Root and canal morphology of human permanent teeth in a Sri Lankan and Japanese population. *Anthropol Sci* 116(2):123–133.
- Plotino G, Tocci L, Grande NM, Testarelli L, Messineo D, Ciotti M, et al. 2013. Symmetry of root and root canal morphology of maxillary and mandibular molars in a white population: a cone-beam computed tomography study in vivo. *J Endod* 39(12):1545–1548.
- Prado-Simón L, Martín-Torres M, Baca P, Olejniczak AJ, Gómez-Robles A, Lapresa M, et al. 2012. Three-dimensional eval-

- uation of root canal morphology in lower second premolars of early and middle pleistocene human populations from atapuerca (Burgos, Spain). *Am J Phys Anthropol* 147:452–461.
- Ramírez-Salomón M, Vega-Lizama E, Tiesler V, Alvarado-Cárdenas G, López-Villanueva M, Sierra-Sosa T, et al. 2014. The C-shaped canal molar: an endodontic-archaeological study of the relationship between Mayan pre-Hispanic and contemporary population of Yucatán. *Int Endod J* 47:1084–1089.
- Rosas A, Martínez-Maza C, Bastir M, García-Tabernero A, Lalueza-Fox C, Hugué R, et al. 2006. Paleobiology and comparative morphology of a Late Neanderthal sample from El Sidrón, Asturias, Spain. *Proc Natl Acad Sci USA* 103: 19266– 19271.
- Seo MS, Park DS. 2004. C-shaped root canals of mandibular second molars in a Korean population: clinical observation and in vitro analysis. *Int Endod J* 37:139–144.
- Sert S, Bayirli GS. 2004. Evaluation of the root canal configurations of the mandibular and maxillary permanent teeth by gender in the Turkish population. *J Endod* 30:391–398.
- Sert S, Şahinkesen G, Topçu FT, Eroğlu SE, Oktay EA. 2011. Root canal configurations of third molar teeth. A comparison with first and second molars in the Turkish population. *Aust Endod J* 37:109–117.
- Sikri VK, Sikri P. 1994. Mandibular premolars: aberrations in pulp space morphology. *Indian J Dent Res* 5:9–14.
- Skidmore AE, Björndal AM. 1971. Root canal morphology of the human mandibular first molar. *Oral Surg Oral Med Oral Pathol* 32:778–784.
- Summerer M, Horst J, Erhart G, Weißensteiner H, Schönherr S, Pacher D, et al. 2014. Large-scale mitochondrial DNA analysis in Southeast Asia reveals evolutionary effects of cultural isolation in the multi-ethnic population of Myanmar. *BMC Evol Biol* 14: 17.
- Torres A, Jacobs R, Lembrechts P, Brizuela C, Cabrera C, Concha G, et al. 2015. Characterization of mandibular molar root and canal morphology using cone beam computed tomography and its variability in Belgian and Chilean population samples. *Imaging Sci Dent* 45:95–101.
- Turner CG II. 1990. Major features of Sundadonty and Sinodonty, including suggestions about East Asians microevolution, population history, and late Pleistocene relationship with Australian Aborigines. *Am J Phys Anthropol* 82: 295–317.
- Valencia de Pablo O, Estevez R, Péix-Sánchez M, Heilborn C, Cohenca C. 2010. Root anatomy and canal configuration of the permanent mandibular first molar: a systematic review. *J Endod* 36: 19 19–1931.
- Vertucci FJ. 1974. Root canal anatomy of the mandibular anterior teeth. *J Am Dent Assoc* 89:369–371.
- Vertucci FJ. 1984. Root canal anatomy of the human permanent teeth. *Oral Sur Oral Med Oral Pathol* 58:589–599.
- Vertucci FJ. 2005. Root canal morphology and its relationship to endodontic procedures. *Endod Topics* 10:3–29.
- Walker RT, Queckenbush LE. 1985. Three-rooted lower first permanent molars in Hong-Kong Chinese. *Br Dent J* 159:298–299.
- Walker RT. 1988a. Root form and canal anatomy of mandibular first molars in a Southern Chinese population. *Endod Dent Traumatol* 4: 19–21.
- Walker RT. 1988b. Root form and canal anatomy of mandibular second molars in a Southern Chinese population. *J Endod* 14:325–329.
- White TD, Folkens PA. 2005. The human bone manual. Amsterdam, The Netherlands: Academic Press.
- Wright T. 2007. The molecular control of and clinical variations in root formation. *Cells Tissues Organs* 186:86–93.
- Yang ZP, Yang SF, Lin YC, Shay JC, Chi CY. 1988. C-shaped root canals in mandibular second molars in a Chinese population. *Dent Traumatol* 4:160–163.